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DESCRIPTION

PROTEIN CAPABLE OF DEPOSITION ONTO EXTRACELLULAR MATRIX

TECHNICAL FIELD

The present invention relates to a protein capable of deposition onto extracellular matrix, which is a partial fragment of developmentally regulated endothelial cell locus-1 (Del-1) protein. The present invention also relates to a method of identifying the site of deposition onto extracellular matrix using the above-described partial fragment, and a method of recovering a molecule of interest (e.g., alkaline phosphatase) fused to Del-1 protein.

BACKGROUND ART

Del-1 (developmentally regulated endothelial cell locus-1) protein (sometimes just referred to as "Del-1" or the "full-length Del-1") is a protein which has EGF (epithelial growth factor)-like domains and discoidin-I-like domains. This protein is an extracellular matrix protein and is known to bind to a protein called αvβ3 integrin receptor or αvβ5 integrin receptor on the surfaces of vascular endothelial cells via the EGF-like domain to thereby promote adhesion of the endothelial cells onto extracellular matrix (Hidai, C. et al., GENES & DEVELOPMENT 12:21-33, 1998).

Recently, a gene encoding the full-length Del-1 has been cloned. It is presumed that the full-length Del-1 is capable of binding, via a part or the entire region thereof, to proteoglycan present in extracellular matrix. A method based on this binding is known in which the full-length Del-1 is expressed; a specific molecule (e.g., a protein or proteoglycan) is bound to the resultant full-length Del-1; and then the molecule bound to the full-length Del-1 (e.g., a protein or proteoglycan) is recovered (see, for example, Japanese Unexamined Patent Publication/PCT No. H11-507527).

Therefore, identification of these binding sites and analysis of the mode of binding are important for recovering molecules of interest and investigating into molecules which bind to the full-length Del-1.

However, since the ability of the full-length Del-1 to deposit onto extracellular matrix is not so high, molecules of interest bound to the full-length Del-1 could not have been recovered sufficiently.

DISCLOSURE OF THE INVENTION

It is an object of the present invention to provide a partial fragment of Del-1 comprising a region capable of efficiently adhering onto extracellular matrix.

As a result of extensive and intensive researches toward the solution of the above problem, the present inventor has found that regions neighboring the discoidin-I-like domains efficiently deposit onto extracellular matrix. Thus, the present invention has been achieved.

The present invention relates to the following.

(1) A protein selected from the following (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 18 or 24;

(b) a protein which comprises the amino acid sequence as shown in SEQ ID NO: 18 or 24 having deletion, substitution or addition of one or several amino acids, and has deposition activity onto extracellular matrix.

(2) A protein selected from the following (a) or (b):

(a) a protein consisting of the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24;

(b) a protein which consists of the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24 having deletion, substitution or addition of one or several amino acids, and has deposition activity onto extracellular matrix.

(3) A protein selected from the following (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 14;

(b) a protein which comprises the amino acid sequence as shown in SEQ ID NO: 14 having deletion, substitution or addition of one or several amino acids, and has inhibitory activity against deposition onto extracellular matrix.

(4) A gene encoding a protein selected from the following (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 18 or 24;

(b) a protein which comprises the amino acid sequence as shown in SEQ ID NO: 18 or 24 having deletion, substitution or addition of one or several amino acids, and has deposition activity onto extracellular matrix.

(5) A gene encoding a protein selected from the following (a) or (b):

(a) a protein consisting of the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24;

(b) a protein which consists of the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24 having deletion, substitution or addition of one or several amino acids, and has deposition activity onto extracellular matrix.

(6) A gene encoding a protein selected from the following (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 14;

(b) a protein which comprises the amino acid sequence as shown in SEQ ID NO: 14 having deletion, substitution or addition of one or several amino acids, and has inhibitory activity against deposition onto extracellular matrix.

(7) A gene comprising a DNA selected from the following (a) or (b):

(a) a DNA comprising the nucleotide sequence as shown in SEQ ID NO: 17 or 23;

(b) a DNA which hybridizes to a DNA comprising a nucleotide sequence complementary to a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 17 or 23 under stringent conditions, and encodes a protein having deposition activity onto extracellular matrix.

(8) A gene comprising a DNA selected from the following (a) or (b):

(a) a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 5, 7, 9, 11, 17 or 23;

(b) a DNA which hybridizes to a DNA consisting of a nucleotide sequence complementary to a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 5, 7, 9, 11, 17 or 23 under stringent conditions, and encodes a protein having deposition activity onto extracellular matrix.

(9) A gene comprising a DNA selected from the following (a) or (b):

(a) a DNA comprising the nucleotide sequence as shown in SEQ ID NO: 13;

(b) a DNA which hybridizes to a DNA comprising a nucleotide sequence complementary to a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 13 under stringent conditions, and encodes a protein having inhibitory activity against deposition onto extracellular matrix.

(10) A recombinant vector comprising the gene according to any one of (4) to (9) above.

(11) A transformant comprising the recombinant vector according to (10) above.

(12) A method of producing a partial fragment of Del-1 protein, comprising culturing the transformant according to (11) above and collecting the partial fragment of Del-1 protein from the resultant culture.

(13) A method of identifying a site in extracellular matrix at which the protein according to any one of (1) to (3) above deposits, comprising reacting the above protein with extracellular matrix.

(14) A reagent for identifying a site of deposition in extracellular matrix, comprising the protein according to any one of (1) to (3) above.

(15) A fusion protein composed of the protein according to any one of (1) to (3) above

linked to a molecule of interest to be expressed.

(16) A drug delivery system comprising the fusion protein according to (15) above.

(17) A gene encoding a fusion protein, wherein the gene according to any one of (4) to (9) above is linked to a gene encoding a molecule of interest to be expressed.

(18) A recombinant vector comprising the gene according to (17) above.

(19) A transformant comprising the recombinant vector according to (18) above.

(20) A method of producing a fusion protein composed of a partial fragment of Del-1 protein and a molecule of interest to be expressed, comprising culturing the transformant according to (19) above and collecting the fusion protein from the resultant culture.

(21) A method of recovering a molecule of interest, comprising allowing the fusion protein according to (15) above to deposit onto extracellular matrix and collecting the molecule of interest.

(22) A method of allowing a molecule of interest to deposit, comprising the following steps:

(a) a step of producing a fusion protein composed of the molecule of interest to be expressed and a partial fragment of Del-1 protein by culturing the transformant according to (19) above; and

(b) a step of allowing the fusion protein to deposit onto extracellular matrix.

(23) A method of recovering a molecule of interest, comprising the following steps:

(a) a step of producing a fusion protein composed of the molecule of interest to be expressed and a partial fragment of Del-1 protein by culturing the transformant according to (19) above;

(b) a step of allowing the fusion protein to deposit onto extracellular matrix; and

(c) a step of cutting off the protein of interest from the fusion protein to thereby collect the molecule of interest.

(24) A method of regulating deposition activity onto extracellular matrix, comprising reacting a fragment within the amino acid sequence as shown in SEQ ID NO: 2 comprising an active center region and a positive regulation region and/or a fragment within the amino acid sequence as shown in SEQ ID NO: 2 comprising an active center region and a negative regulation region with extracellular matrix.

(25) The method according to (24) above, wherein the amino acid sequence of the active center region is as shown in SEQ ID NO: 4.

(26) The method according to (24) above, wherein the amino acid sequence of the positive regulation region is as shown in SEQ ID NO: 20.

(27) The method according to (24) above, wherein the amino acid sequence of the negative

regulation region is as shown in SEQ ID NO: 22.

According to the present invention, *Del-1* partial fragments are provided. Since the proteins expressed from these *Del-1* partial fragments have deposition activity onto extracellular matrix, use of the *Del-1* partial fragment allows a molecule of interest linked to the protein expressed from the *Del-1* partial fragment to deposit onto extracellular matrix efficiently. Also, it is possible to recover or remove the molecule of interest by means of this deposition.

By allowing a molecule of interest to deposit onto extracellular matrix using the *Del-1* partial fragment of the invention, it is possible to concentrate and localize the molecule of interest in a target tissue. In particular, by preventing the molecule of interest from flowing into plasma, it is possible to prevent the migration of that molecule into other tissues.

The *Del-1* partial fragments of the invention include those fragments which express proteins having a function of inhibiting the deposition onto extracellular matrix. Therefore, by increasing/decreasing the deposition activity with a combination of a fragment having deposition activity and a fragment having deposition inhibitory activity, it is possible to control the recovery, removal, concentration, etc. of a molecule of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing an outline of the nucleotide sequences of the *Del-1* partial fragments of the invention and the results of measurement of deposition activities of individual fragments using alkaline phosphatase activity.

Fig. 2 is a diagram showing the deposition activities of the Del-1 partial fragments of the invention.

Fig. 3 is a diagram showing AP/Lac ratios in plasma samples taken from individual livers.

Fig. 4 is a diagram showing AP/Lac ratios in hepatic tissue samples taken from individual livers.

Fig. 5 is a diagram showing the results of alkaline phosphatase staining of hepatic tissue samples taken from individual livers.

Fig. 6 is a diagram showing the results of Western blotting.

Fig. 7 is a diagram showing the results of alkaline phosphatase recovery.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention relates to partial fragments of the full-length Del-1 protein

which comprise a region that specifically binds to extracellular matrix. Specifically, the present invention relates to Del-1 deposition proteins and Del-1 deposition inhibitory proteins (sometimes, simply referred to as "Del-1 partial fragments"). The Del-1 partial fragments of the invention are obtained by truncating the full-length Del-1 into fragments with varied lengths and characterized by having deposition activity onto extracellular matrix.

The Del-1 partial fragment of the invention comprises amino acids encoded by a region of the full-length *Del-1* gene (SEQ ID NO: 1) spanning at least from position 1270 to position 1662 (corresponding to an amino acid sequence from position 218 to position 348 of the amino acid sequence as shown in SEQ ID NO: 2). The nucleotide sequence of this region is shown in SEQ ID NO: 3 and the amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 4. The *Del-1* partial fragment of the invention comprising the above-described region has the nucleotide sequence as shown in SEQ ID NO: 5, 7, 9, 11, 13, 15 or 17. The amino acid sequences encoded by these nucleotide sequences are shown in SEQ ID NOS: 6, 8, 10, 12, 14, 16 and 18, respectively.

It is presumed that the Del-1 partial fragment described above is capable of binding to proteoglycan in view of the amino acid sequence encoding the partial fragment.

For detecting the full-length Del-1 protein or Del-1 partial fragments, a method using alkaline phosphatase is employed. Briefly, by allowing cells to express a fusion protein composed of the full-length Del-1 protein to which alkali phosphatase is fused to the N terminus by genetic recombination, alkaline phosphatase activity can be confirmed in culture supernatant as well as extracellular matrix.

In the present invention, in addition to the above-described detection method using alkaline phosphatase, it is also possible to use Western blotting for the detection of Del-1 partial fragments, etc. Specifically, a nucleotide sequence encoding a fusion protein composed of alkaline phosphatase and the full-length Del-1 or a Del-1 partial fragment is introduced into cos7 cells. The cells are cultured for a specific period of time, and then the culture medium and extracellular matrix are collected and subjected to Western blotting for detection. As controls, laminin and albumin may be used, for example. In the Western blotting, in order to improve the detection sensitivity for the Del-1 protein or Del-1 partial fragment in the culture supernatant, the volume of culture medium used in the method may be increased and the protein may be concentrated.

Although either of the above detection methods may be used, the method using alkaline phosphatase is preferable.

In the present invention, the full-length Del-1 (which is known) was truncated by various methods to prepare Del-1 partial fragments of the invention. The resultant partial

fragments were detected by the above-described detection method using alkaline phosphatase and subjected to Western blotting to examine the ability to deposit onto extracellular matrix. Further, the site of deposition of the Del-1 partial fragment onto extracellular matrix was identified; and immobilization of the Del-1 partial fragment onto a specific site in the living body was performed. Further, the expression product of a gene of interest was recovered using the Del-1 partial fragment.

Hereinbelow, embodiments of the present invention will be described specifically.

1. DNAs encoding Del-1 Partial Fragments

Del-1 partial fragments can be obtained by truncating the DNA encoding the full-length Del-1 protein into various lengths and then expressing these truncated DNAs.

The full-length *Del-1* gene may be cloned by the known method (Hidai, C. et al., GENES & DEVELOPMENT 12:21-33, 1998). Briefly, an exon is obtained from a genomic library by exon trapping. Using this exon, cDNA of *Del-1* can be cloned.

For example, a fragment from a genomic clone is inserted into a splicing vector to thereby cause splicing at the time of transcription of mRNA. Subsequently, the spliced mRNA is reverse-transcribed and amplified, followed by sequencing of the exon.

The resultant exon is used as a probe to probe a cDNA library for the DNA of interest, or used in designing gene specific primers for 5'-RACE or 3'-RACE. RACE may be performed with commercial kits (e.g., Marathon™ cDNA Amplification Kit; Clontech).

The determination of the nucleotide sequence of cDNA may be performed by any of known methods. Usually, sequencing is performed with an automated DNA sequencer.

The thus obtained nucleotide sequence of the full-length cDNA is shown in SEQ ID NO: 1. The amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 1 is shown in SEQ ID NO: 2.

One of the truncated Del-1 partial fragments of the invention comprises an amino acid sequence spanning from positions 1 to 348 of the amino acid sequence as shown in SEQ ID NO: 2. This partial fragment can be obtained by serially deleting a DNA having the nucleotide sequence as shown in SEQ ID NO: 1 from the 3' end with exonuclease III and mung bean nuclease. The 3' terminal DNA deleted is determined by the reaction time of exonuclease III. In this method, a commercial enzyme (e.g., Exonuclease III; Takara Bio) may be used.

A schematic diagram showing the full-length *Del-1* (Del-1 major), truncated *Del-1* partial fragments of the invention and amino acid sequences affecting the deposition activities of these partial fragments is shown in the left upper part of Fig. 1.

In Fig. 1, the following partial fragments have the following amino acid sequences in the amino acid sequence as shown in SEQ ID NO: 2. CY has the amino acid sequence of a region spanning from positions 218 to 348 (SEQ ID NO: 4); 4-1 has the amino acid sequence of a region spanning from positions 1 to 348 (SEQ ID NO: 6); 4-14 has the amino acid sequence of a region spanning from positions 1 to 368 (SEQ ID NO: 10); 4-13 has the amino acid sequence of a region spanning from positions 1 to 385 (SEQ ID NO: 12); CB has the amino acid sequence of a region spanning from positions 218 to 480 (SEQ ID NO: 14); and XY has the amino acid sequence of a region spanning from positions 123 to 348 (SEQ ID NO: 18).

DNAs encoding these Del-1 partial fragments (designated "DNAs of the invention") have the following nucleotide sequences in the nucleotide sequence as shown in SEQ ID NO: 1. CY has the nucleotide sequence of a region spanning from positions 1270 to 1662 (393 bp, SEQ ID NO: 3); 4-1 has the nucleotide sequence of a region spanning from positions 619 to 1662 (1044 bp, SEQ ID NO: 5); 4-14 has the nucleotide sequence of a region spanning from positions 619 to 1722 (1104 bp, SEQ ID NO: 9); 4-13 has the nucleotide sequence of a region spanning from positions 619 to 1773 (1155 bp, SEQ ID NO: 11); CB has the nucleotide sequence of a region spanning from positions 1270 to 2058 (789 bp, SEQ ID NO: 13); and XY has the nucleotide sequence of a region spanning from positions 985 to 1662 (678 bp, SEQ ID NO: 17).

Further, human XY (SEQ ID NO: 24) in human full-length Del-1 corresponding to mouse fragment XY (SEQ ID NO: 18) was also measured for its deposition activity. The DNA encoding human XY has the nucleotide sequence as shown in SEQ ID NO: 23.

Although not shown in Fig. 1, 4-15 and DE are also truncated Del-1 partial fragments of the invention; 4-15 has the amino acid sequence of a region spanning from 1 to 365 of the amino acid sequence as shown in SEQ ID NO: 2 (SEQ ID NO: 8) and DE has the amino acid sequence of a region spanning from 218 to 319 of the amino acid sequence as shown in SEQ ID NO: 2 (SEQ ID NO: 16). DNAs encoding these amino acid sequences have the nucleotide sequence of a region spanning from positions 619 to 1713 of the nucleotide sequence as shown in SEQ ID NO: 1 (1095 bp, SEQ ID NO: 7) for 4-15 and the nucleotide sequence of a region spanning from positions 1270 to 1575 of the nucleotide sequence as shown in SEQ ID NO: 1 (306 bp, SEQ ID NO: 15) for DE.

In Fig. 1, XC has the amino acid sequence of a region spanning from positions 123 to 217 (SEQ ID NO: 20) and YB has the amino acid sequence of a region spanning from positions 349 to 480 (SEQ ID NO: 22) as an amino acid sequence improving or reducing the deposition activity of the Del-1 partial fragment of the invention. DNAs encoding these

amino acid sequences have the nucleotide sequence of a region spanning from positions 985 to 1269 (285 bp, SEQ ID NO: 19) for XC and the nucleotide sequence of a region spanning from positions 1663 to 2058 (396 bp, SEQ ID NO: 21) for YB.

Further, the partial fragments of the present invention comprise CY represented by an amino acid sequence spanning at least from position 218 to position 348 (SEQ ID NO: 4) of the amino acid sequence as shown in the above-mentioned SEQ ID NO: 2. In one embodiment of the invention, the partial fragment of the invention comprises a protein in which a plurality of the amino acid sequences spanning at least from position 218 to position 348 (SEQ ID NO: 4) of the amino acid sequence as shown in the above-mentioned SEQ ID NO: 2 are connected. This region is the center region having deposition activity onto extracellular matrix. The above-described CY is encoded by a region spanning from positions 1270 to 1662 (SEQ ID NO: 3) of the nucleotide sequence as shown in SEQ ID NO: 1.

The amino acid sequence as shown in SEQ ID NO: 20 (XC) improves deposition activity onto extracellular matrix and is a positive regulation region for the deposition activity. On the other hand, the amino acid sequence as shown in SEQ ID NO: 22 (YB) reduces deposition activity onto extracellular matrix and is a negative regulation region for the deposition activity. The "positive regulation region" means a region which does not cause deposition activity by itself but is capable of causing deposition activity when the center region CY is included in the relevant fragment. The "negative regulation region" means a region whose presence, as a whole or as a part, causes reduction in deposition activity regardless of the presence of center region CY or positive regulation region XC, resulting in increase in soluble fraction.

The regions contained in the Del-1 partial fragments of the invention are summarized in the following Table 1.

Table 1

Designation	Region*		Type	SEQ ID NO:
Full-length *Del-1*			DNA	1
Full-length Del-1	619-2061		Protein	2
CY	1270-1662	Center region	DNA	3
CY	218-348	Center region	Protein	4
4-1	619-1662	Comprising center region + positive regulation region	DNA	5
4-1	1-348	Comprising center region + positive regulation region	Protein	6
4-15	619-1713	Center region + positive regulation region	DNA	7
4-15	1-365	Comprising center region + positive regulation region	Protein	8
4-14	619-1722	Center region + positive regulation region	DNA	9
4-14	1-368	Center region + positive regulation region	Protein	10
4-13	619-1773	Center region + positive regulation region	DNA	11
4-13	1-385	Center region + positive regulation region	Protein	12
CB	1270-2058	Center region + negative regulation region	DNA	13
CB	218-480	Center region + negative regulation region	Protein	14
DE	1270-1575		DNA	15
DE	218-319		Protein	16
XY	985-1662	Center region + positive regulation region	DNA	17
XY	123-348	Center region + positive regulation region	Protein	18
XC	985-1269	Positive regulation region	DNA	19
XC	123-217	Positive regulation region	Protein	20
YB	1663-2058	Negative regulation region	DNA	21
YB	349-480	Negative regulation region	Protein	22
human XY		Center region + positive regulation region	DNA	23
human XY		Center region + positive regulation region	Protein	24

* Regions are expressed with nucleotide positions for DNAs and with amino acid positions for proteins.

Once the regions to be included in a partial fragment are determined, primers are designed so that those regions are amplified. Then, a DNA encoding the partial fragment can be readily obtained by PCR using the DNA encoding Del-1 as a template.

In the present invention, it should be noted that as long as the protein consisting of the above-described amino acid sequence for the Del-1 partial fragment has deposition activity onto extracellular matrix, the amino acid sequence may have mutations, such as deletion, substitution or addition, in at least one, preferably one or several amino acids.

For example, one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be deleted from the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24; one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be added to the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24; and one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be substituted with other amino acids in the amino acid sequence as shown in SEQ ID NO: 6, 8, 10, 12, 18 or 24. Therefore, genes encoding proteins comprising the above mutation-introduced amino acid sequences are also included in the gene of the invention as long as the proteins have deposition activity onto extracellular matrix.

It should be also noted that as long as the protein consisting of the above-described amino acid sequence for the Del-1 partial fragment has a function to inhibit deposition activity onto extracellular matrix, the amino acid sequence may have mutations, such as deletion, substitution or addition in at least one, preferably one or several amino acids.

For example, one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be deleted from the amino acid sequence as shown in SEQ ID NO: 14 which represents CB region; one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be added to the amino acid sequence as shown in SEQ ID NO: 14; and one or several amino acids (e.g., 1 to 10, preferably 1 to 5 amino acids) may be substituted with other amino acids in the amino acid sequence as shown in SEQ ID NO: 14. Therefore, genes encoding proteins comprising the above mutation-introduced amino acid sequences are also included in the gene of the invention as long as the proteins have activity to inhibit deposition onto extracellular matrix.

Introduction of the above-described mutations such as deletion, substitution or addition may be performed with a kit utilizing site-directed mutagenesis techniques, e.g., GeneTailor™ Site-Directed Mutagenesis System (Invitrogen) or TaKaRa Site-Directed Mutagenesis System (Mutan-K, Mutan-Super Express Km; Takara Bio).

Further, in the present invention, a DNA which is hybridizable to a DNA consisting of a nucleotide sequence complementary to the DNA encoding the above-described Del-1 partial fragment (SEQ ID NO: 5, 7, 9, 11, 17 or 23) under stringent conditions and encodes a protein having binding activity to extracellular matrix is also included in the gene of the invention. Stringent conditions means, for example, salt (sodium) concentration is 150-900 mM and temperature is 55-75°C; preferably, salt (sodium) concentration is 150-200 mM and temperature is 60-70°C.

Further, in the present invention, a DNA which is hybridizable to a DNA consisting of a nucleotide sequence complementary to the DNA encoding the above-described Del-1 partial fragment (SEQ ID NO: 13) under stringent conditions and encodes a protein having activity to inhibit deposition onto extracellular matrix is also included in the gene of the invention.

The term "extracellular matrix" (ECM) refers to a biological structure present outside of cells in animal tissues and means an assembly of biopolymers which were synthesized within cells and secreted/accumulated outside of the cells. Major components of extracellular matrix are collagen, elastin, proteoglycan, glycosaminoglycan and sugar proteins. "Deposition activity" means the activity of the entire region or a partial fragment of Del-1 binding to extracellular matrix. Some partial fragments have higher deposition activity than the full-length Del-1, and some have lower deposition activity than the full-length Del-1. Some fragments shorter than the full-length Del-1 but having equivalent deposition activity are also included. The "activity to inhibit deposition onto extracellular matrix" means the activity of reducing deposition activity and thus increasing soluble fraction, which is caused by the presence of a negative regulation region regardless of the presence of center region CY or positive regulation region XC. Measurement of deposition activity or activity to inhibit deposition onto extracellular matrix may be performed, for example, as described below.

Briefly, a DNA encoding a marker such as alkaline phosphatase is linked to the DNA of the invention. The resultant DNA is introduced into a specific cell (e.g., cos7 cells, CHO cells, NIH3T3 cells, etc.), which is then cultured. After the culture supernatant and cells are removed from the culture dish, the substrate of alkaline phosphatase is added to the extracellular matrix remaining in the dish for color development to thereby measure deposition activity. Since a marker (alkaline phosphatase) is linked to the Del-1 partial fragment, when the Del-1 partial fragment deposits onto extracellular matrix, it is possible to measure the binding activity and also to identify the site of binding using the marker as an indicator. For example, when a soluble alkaline phosphatase substrate is used, the substrate

develops a color (e.g., yellow). Thus, deposition activity can be easily determined by measuring absorbance at a specific wavelength. Alternatively, when an alkaline phosphatase of deposition property is used, the site of deposition develops a color (e.g., purple). Thus, the deposition site can be easily identified by microscopic observation or the like.

The marker useful in the invention is not limited to alkaline phosphatase. GFP or a variation thereof, a tag such as myc or His, GST protein, an isotope, a biotinylated protein or the like may also be used. Alternatively, it is possible to perform an assay using a reporter gene such as chloramphenicol acetyltransferase (CAT) gene, luciferase gene, or β galactosidase gene.

2. Preparation of Recombinant Vectors and Transformants Comprising the DNA of the Invention

(1) Preparation of Recombinant Vectors Comprising the DNA

Recombinant vectors comprising the DNA of the invention can be obtained by linking (introducing) the DNA of the invention to an appropriate vector. The vector to which the DNA of the invention is to be inserted is not particularly limited as long as it is capable of replication in a host. For example, plasmid DNA, phage DNA, virus or the like may be used.

As plasmid DNA, *Escherichia coli*-derived plasmids, *Bacillus subtilis*-derived plasmids, yeast-derived plasmid and the like may be enumerated. As phage DNA, λ phage and the like may be enumerated. As virus, adenovirus, retrovirus and the like may be enumerated.

The vector of the invention may contain, if desired, cis elements such as enhancers, splicing signals, poly(A) addition signals, selection markers, ribosome binding sequences (SD sequences) or the like in addition to the DNA of the invention. As the selection marker, dihydrofolate reductase gene, ampicillin resistance gene, neomycin resistance gene or the like may be enumerated.

(2) Preparation of Transformants

The transformant of the invention may be obtained by introducing the recombinant vector of the invention into a host so that the gene of interest can be expressed. The host is not particularly limited as long as it can express the DNA of the invention. Specific examples of hosts which may be used in the invention include well-known bacteria, yeasts, animal cells and insect cells. Alternatively, experimental animals such as mouse, domestic

animals such as pig, plants such as rice or maize, and the like may be used.

When a bacterium is used as a host, the recombinant vector of the invention is capable of autonomous replication in the host and, at the same time, may also comprise a promoter, a ribosome binding sequence, the DNA of the invention and a transcription termination sequence. Specific examples of bacteria which may be used in the invention include *Escherichia coli* and *Bacillus subtilis*. As a promoter, trp promoter, lac promoter, PL promoter, PR promoter or the lime may be used. The method of introducing the recombinant vector into a bacterium is not particularly limited. For example, the calcium ion method or electroporation may be used.

When a yeast is used as the host, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or the like may be used. A promoter which may be used in this case is not particularly limited. Any promoter may be used as long as it can direct the expression of the DNA in yeast. For example, gal1 promoter, gal10 promoter, heat shock protein promoter, MFα1 promoter, PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, or the like may be enumerated. As a method of introducing the recombinant vector into the yeast, electroporation, the spheroplast method, the lithium acetate method, or the like may be enumerated.

When an animal cell is used as the host, simian cells (cos7 cells), Vero cells, Chinese hamster ovary cells (CHO cells), mouse L cells, rat GH3 cells, human FL cells or HEK293 cells, or the like may be used. As a promoter, SRα promoter, SV40 promoter, LTR promoter, β-actin promoter, or the like may be used. As a method for introducing the recombinant vector into an animal cell, electroporation, the calcium phosphate method, lipofection, or the like may be enumerated.

When an insect cell is used as the host, Sf9cells, Sf21 cells, or the like may be used. As a method for introducing the recombinant vector into an insect cell, the calcium phosphate method, lipofection, electroporation, or the like may be used.

Gene transfer into animals or plants may be performed, for example, by methods using a virus vector or lipofection. It is also possible to introduce a gene into germ line cells or ES cells to thereby create genetically modified animals.

3. Production of the Del-1 Partial Fragment of the Invention

The Del-1 partial fragment of the invention can be obtained by culturing or breeding the above-described transformant and recovering the fragment from the resultant culture or breeding product. The term "culture" means any of the following materials: culture supernatant, cultured cells, cultured microorganisms, or disrupted materials from

cells or microorganisms. The term "breeding product" means any of the following materials: bodies, tissues, secreted materials or excreta of animals or plants, or products obtained by processing these materials.

Cultivation of the transformant of the invention is carried out in accordance with conventional methods commonly used for culturing hosts.

As a medium to culture the transformant obtained from a microorganism host such as bacterium or yeast, either a natural of synthetic medium may be used as long as it contains carbon sources, nitrogen sources and inorganic salts assimilable by the microorganism and is capable of efficient cultivation of the transformant.

As carbon sources, carbohydrates such as glucose, fructose, sucrose, starch; organic acids such as acetic acid, propionic acid; and alcohols such as ethanol and propanol may be used.

As nitrogen sources, ammonia; ammonium salts of inorganic or organic acids such as ammonium chloride, ammonium sulfate, ammonium acetate, ammonium phosphate; Peptone; meat extract; corn steep liquor and the like may be used.

As inorganic substances, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, iron(II) sulfate, manganese sulfate, copper sulfate, calcium carbonate and the like may be used.

Usually, cultivation is carried out under aerobic conditions (such as shaking culture or aeration agitation culture) at 37°C for 12 to 24 hours. Adjustment of the pH is carried out using an inorganic or organic acid, an alkali solution or the like.

When a microorganism transformed with an expression vector containing an inducible promoter is cultured, an inducer may be added to the medium, if necessary. For example, when a microorganism transformed with an expression vector containing lac promoter is cultured, isopropyl-β-D-thiogalactoside (IPTG) or the like may be added to the medium.

As a medium to culture a transformant obtained from an animal cell as a host, commonly used RPMI-1640 medium or DMEM medium, or one of these media supplemented with fetal bovine serum, etc. may be used.

Usually, cultivation is carried out in the presence of 5% CO_2 at 37°C for 1 to 4 days. During the cultivation, antibiotics such as kanamycin or penicillin may be added to the medium, if necessary.

After the cultivation, the protein of the invention is extracted by disrupting the microorganisms or cells when the protein is produced within the microorganisms or cells. When the protein of the invention is produced outside the microorganisms or cells, the

culture medium is used as it is, or subjected to centrifugation to remove the microorganisms or cells. Thereafter, the resultant supernatant is subjected to conventional biochemical techniques used for isolating/purifying proteins. These techniques include ammonium sulfate precipitation, gel chromatography, ion exchange chromatography and affinity chromatography, and may be used independently or in an appropriate combination. Thus, the Del-1 partial fragment of the invention can be isolated/purified from the above-mentioned culture.

When an animal (experimental animal or domestic animal such as mouse, rat, rabbit, goat or bovine) or a plant is used as a transformant, they may require special breeding or culturing method such as ascetic environment or special feeds. If the transformant is one of the animals mentioned above, the Del-1 partial fragment of the invention may be isolated/purified from meat, eggs, hair, breastmilk, feces or the like of the transformant by using common biochemical techniques (such as ammonium sulfate precipitation, gel chromatography, ion exchange chromatography and affinity chromatography) independently or in combination.

When the transformant is a plant, the Del-1 partial fragment of the invention may be isolated/purified not only from leaves, flowers, fruits and roots of the transformant but also from the soil or water used for the cultivation, by using common biochemical techniques (such as ammonium sulfate precipitation, gel chromatography, ion exchange chromatography and affinity chromatography) independently or in combination.

In the present invention, synthesis of the Del-1 partial fragment by *in vitro* translation may be employed. Two methods may be available for the synthesis. One is a method using RNA as a template and the other is a method using DNA as a template (transcription/translation). As a template DNA, the above-described DNA having a promoter and a ribosome binding site upstream of the translation start point, or a DNA in which necessary elements for transcription (e.g., promoter) are integrated upstream of the translation start point may be used. As an *in vitro* translation system, a commercial system such as Expressway™ system (Invitrogen) or TNT system (registered trademark; Promega) may be used. After translation of the Del-1 partial fragment by an *in vitro* translation system, the fragment of interest can be isolated/purified by using the above-described biochemical methods independently or in combination.

4. Recovery of the Expression Product of the Gene of Interest

A cell system or an animal or plant expressing the Del-1 partial fragment and a molecule of interest may be used to recover the molecule of interest (i.e., expression product

of the gene of interest) (for example, protein, antibody, peptide, natural or synthetic compound, other cell, or soluble molecule) by allowing expression of the gene of interest. Alternatively, the Del-1 partial fragment may be used directly.

The method of recovering a molecule of interest will be described below. First, a fusion protein in which a molecule of interest is bound to the Del-1 partial fragment is prepared. Briefly, a DNA encoding the molecule of interest and a DNA encoding the Del-1 partial fragment are linked, and the resultant DNA is linked to an appropriate vector. This vector is introduced into an appropriate host cell, which is then cultured to thereby produce the fusion protein in which the molecule of interest is linked. Methods of linking to the vector, introducing into the cell, culturing the transformant cell, and breeding of the transformant are as described in the preceding sections 2 and 3.

When the transformant cell is used, the entire region or a part of the Del-1 partial fragment in the fusion protein deposits onto extracellular matrix spreading on the culture dish. Therefore, even when the culture supernatant and cells have been removed after the cultivation, the fusion protein remains in the culture dish in a state of deposition onto extracellular matrix. Thus, it is possible to recover the molecule of interest by mechanically scraping the extracellular matrix onto which the fusion protein is depositing. Alternatively, it is possible to recover the molecule of interest alone by inserting in advance a recognition sequence of a specific enzyme (e.g., Factor Xa) between the nucleotide sequence of the molecule of interest and the nucleotide sequence of the Del-1 partial fragment and then using the enzyme. It is also possible to recover the molecule of interest into a solution by adding a negative regulation region to the Del-1 partial fragment.

Here, it is necessary to label the Del-1 partial fragment in order to identify and isolate the molecule of interest from the fusion protein in which the Del-1 partial fragment and the molecule of interest are linked. It is possible to label the Del-1 partial fragment with an enzyme such as alkaline phosphatase or horse radish peroxidase; or a reagent such as a fluorescent label containing fluoresceine isothiocyanate (FITC), phycocyanin or rhodamine.

Since the Del-1 partial fragment of the invention has deposition activity onto extracellular matrix, the partial fragment is applicable to binding assay, affinity chromatography, immunoprecipitation, Western blotting, and the like.

Identification of polypeptides of interest to be expressed which are capable of binding to the Del-1 partial fragment can also be performed by screening a peptide library with a recombinant Del-1 partial fragment.

Briefly, the above-described fusion protein which is labeled is incubated with a

random peptide library to thereby bind the Del-1 partial fragment to peptides in the library. Subsequently, the library is washed to remove unbound polypeptides. To wells containing a substrate for alkaline phosphatase or peroxidase (e.g., 5-bromo-4-chloro-3-indolylphosphate (BCIP) or 3,3'-diaminobenzidine (DAB)), peptides of the library are added and incubated for several minutes. Then, alkaline phosphatase or the like develops a color. Thus, molecules of interest can be easily identified and isolated.

In the case of the transformant being an animal or plant, when the above-described fusion protein is expressed in a specific site of the animal or plant, the Del-1 partial fragment of the invention deposits onto extracellular matrix to thereby concentrate the protein of interest in that tissue. Therefore, the molecule of interest can be efficiently recovered and used by directly eating the relevant agricultural or livestock product or by extracting biochemically.

5. Identification of Deposition Sites on Extracellular Matrix

As described in the preceding section 1, the Del-1 partial fragment of the invention has deposition activity onto extracellular matrix. By using a deposition marker, it is possible to observe visually the deposition site of the Del-1 partial fragment of the invention on extracellular matrix.

Therefore, the Del-1 partial fragment of the invention is useful as a reagent for identifying the deposition site on extracellular matrix and can be included in an extracellular matrix deposition site identification kit together with a marker, a color development substrate, an antibody to the marker, etc.

6. Immobilization of Biologically Active Substances at Specific Sites in the Living Body

When a fusion protein composed of a molecule of interest and the Del-1 partial fragment of the invention is expressed in a specific tissue, the molecule of interest is immobilized at the specific site and does not migrate to other sites. As a result, the molecule of interest is concentrated at that site.

Therefore, the nucleotide sequence encoding the Del-1 partial fragment of the invention can be used, in combination with a promoter sequence specific to an appropriate cell, tissue or organ, as a vector for expressing a molecule of interest in a specific tissue and immobilizing, localizing and concentrating the molecule.

Further, as a result of staining with BCIP, it was found that extracellular alkaline phosphatase activity is present in extracellular matrix (Example 2).

This means that the partial fragment of Del-1 protein has much higher ability to

deposit onto extracellular matrix than the full-length Del-1 protein, and has an effect of immobilizing other proteins such as alkaline phosphatase in extracellular matrix.

7. Modification of Artifacts with Biologically Active Substances

It is possible to allow a biologically active substance to deposit onto an artifact without damaging its biological function, by culturing on the artifact *E. coli* or other cells producing a fusion protein composed of the biologically active substance and the Del-1 partial fragment of the invention. For example, the results of Fig. 2 show that a culture dish (an artifact) has been modified with alkaline phosphatase (a biologically active substance). This is applicable to modification of membranes for hemodialysis and artificial materials for implantation.

8. Regulation of Deposition Activity and Drug Delivery System

When linked to a molecule of interest, the Del-1 partial fragment of the invention is capable of allowing the molecule to deposit onto extracellular matrix. Further, it is possible to artificially regulate the deposition activity of the Del-1 partial fragment of the invention by using a positive regulation region and a negative regulation region. For example, it is possible to change the degree of deposition activity by the presence or absence of YB region or XC region as shown in Fig. 1, or by appropriately changing the lengths of these regions (see, for example, 4-8, 4-13, 4-1 and XY in Fig. 1). Specifically, a fragment comprising the active center region CY (SEQ ID NOS: 4 and 5) and the positive regulation region (SEQ ID NOS: 19 and 20), a fragment comprising the active center region CY (SEQ ID NOS: 4 and 5) and the negative regulation region (SEQ ID NOS: 21 and 22) or both of these fragments may be reacted with extracellular matrix for positive or negative regulation, to thereby obtain deposition activities of varied strengths, wherein all of these fragments are in the amino acid sequence as shown in SEQ ID NO: 2. Therefore, when the molecule of interest is a protein having a specific pharmacological effect, the fusion protein of the invention may be used as a drug delivery system (DDS). For example, a gene encoding a fusion protein composed of fragment 4-1 comprising the center region and the positive regulation region and an enzyme that converts a precursor of an anticancer agent into the anticancer agent is transferred into cancer tissues in advance. Subsequently, a large dose of the precursor is administered. Then, a higher drug concentration is achieved in cancer tissues than normal tissues. After the treatment, by introducing a gene encoding fragment CB (SEQ ID NOS: 13 and 14) comprising the negative regulation region, the gene product of the previously introduced gene is released into blood and becomes capable of removal by hemodialysis or the like.

EXAMPLES

Hereinbelow, the present invention will be described more specifically with reference to the following Examples. However, the present invention is not limited to these Examples.

[EXAMPLE 1] Preparation of Del-1 Partial Fragments

RNA was extracted from mouse embryos 9 to 12 days after fertilization using TRIzol (Invitrogen). Using the resultant RNA as a template, reverse transcription was performed to prepare cDNA. The nucleotide sequence from positions 697 to 2089 corresponding to the amino acid sequence as shown in SEQ ID NO: 2 with its signal peptide sequence deleted was amplified by PCR. A restriction enzyme recognition sequence was added at the 5' end of the primer so that the above nucleotide sequence can be inserted into a vector after PCR amplification. The nucleotide sequences of the primers are as described below.

Forward primer: AAA GAT CTA ACC CGA ACC CCT GTG AA (SEQ ID NO: 25)
Reverse primer: AAC TCG AGC ATT GTG GGA TGT GCG (SEQ ID NO: 26)

PCR was performed using a reaction solution with the following composition for 35 cycles at 94°C, 30 seconds; 62°C, 30 seconds; 72°C, 1 minute and 30 seconds.

Composition of the reaction solution (in 50 μl):	
cDNA produced by a reverse transcriptase	5 μl
Primers	1 μM for each
dNTPs	0.5 mM for each
Polymerase	2 units
Buffer	10 mM Tris-HCl (pH 8.3)
	50 mM KCl
	1.5 mM $MgCl_2$

The resultant PCR product was treated with restriction enzymes Bgl II and XhoI, and then ligated to plasmid pATtag-5 (Funakoshi). The thus prepared plasmid was digested with Xho I and then treated with Exonuclease III (Takara Bio) for 10 seconds to 2 minutes, to thereby prepare Del-1 partial fragments with varied lengths shown in Fig. 1 (4-8, 4-13, 4-14, 4-1, 4-11, 2-6, Del-1 minor, 1-1 and 2-3). Also, Del-1 partial fragments with varied lengths shown in Fig. 1 (CB, CY, YB, XY, XC, human XY, and AP only) and Del-1 partial fragments not shown in Fig. 1 (FB: positions 1576-2059 of the nucleotide sequence as shown in SEQ ID NO: 1; 4-15: SEQ ID NO: 8; and CE: SEQ ID NO: 16) were prepared by

PCR.

[EXAMPLE 2] Deposition Activity of Del-1 Partial Fragments onto Extracellular Matrix

(1) Of the partial fragments prepared in Example 1, 4-8, 4-13, 4-14, 4-1, 4-11, 2-6, Del-1 minor, 1-1 and 2-3 were ligated to plasmid pAPtag-5 (Funakoshi) and introduced into cos7 cells. Three days after the introduction, the culture supernatant, cells and extracellular matrix were collected. First, after collecting the culture supernatant, 0.05% EDTA-containing PBS was added to the culture dish and incubated. This operation allows cells to peel off from the bottom of the culture dish and to become collectable. As a result, the extracellular matrix is left on the bottom of the culture dish. Thus, alkaline phosphatase activities in these fractions were detected. As controls, samples of the wild-type, full-length Del-1 (AP4Del-1) and the medium alone were prepared, followed by detection of alkaline phosphatase activities therein. Alkaline phosphatase activity was determined as a ratio of the activity in extracellular matrix to the activity in culture supernatant (AP activity ratio; ECM/Medium) and shown in a graph at the right side of Fig. 1.

From Fig. 1, it can be seen that 4-1, 4-8, 4-14 and 4-13 have stronger activity than the wild-type Del-1 (Del-1 major); that 4-11 and 2-6 have lower activity than Del-1 major; and that Del-1 minor has little activity.

In order to examine the center region of deposition activity, CB (positions 1270-2058 of the nucleotide sequence as shown in SEQ ID NO: 1), CY, YB, XY, XC, human XY and AP only were expressed, and alkaline phosphatase activities therein were measured in the same manner as described above.

As a result, XY and human XY have higher alkaline phosphatase activity than the wild-type full-length Del-1, and CB and CY have some alkaline phosphatase activity. On the other hand, no alkaline phosphatase activity was recognized in XC and YB.

From these results, it was believed that the active center region is CY encoded by SEQ ID NO: 3 (a region spanning from positions 1270 to 1662 of the nucleotide sequence as shown in SEQ ID NO: 1) which corresponds to a region spanning from positions 218 to 348 of the amino acid sequence as shown in SEQ ID NO: 2.

XY, which consists of CY and XC ligated, has deposition activity about 10 times higher that that of CY (active center region) alone. XC alone has little deposition activity. Therefore, it was believed that XC is a positive regulation region for deposition activity which improves deposition activity onto extracellular matrix.

On the other hand, the deposition activity of CB, which consist of CY and YB ligated, is reduced to about 0.5 times the activity of the active center region CY alone.

Therefore, it was believed that YB is a negative regulation region for deposition activity which decreases deposition activity onto extracellular matrix.

(2) Further, from the Del-1 partial fragments prepared in Example 1, Del-1 minor (positions 619-1271 of the nucleotide sequence as shown in SEQ ID NO: 1) or 4-1 was ligated to plasmid pAPtag-5 (Funakoshi) and introduced into cos7 cells. Three days after the introduction, the culture supernatant, cells and extracellular matrix were collected. First, after collecting the culture supernatant, 0.05% EDTA-containing PBS was added to the culture dish and incubated. This operation allows cells to peel off from the bottom of the culture dish and to become collectable. As a result, the extracellular matrix is left on the bottom of the culture dish. Thus, alkaline phosphatase activities in these fractions were detected.

The results are shown in Fig. 2. In Fig. 2, panels A to D show the results from those samples prepared using Del-1 minor; and panels E to H show the results from those samples prepared using 4-1. Panels A and E show the results of staining cells with an alkaline phosphatase substrate of deposition property (BCIP). Panels B and F show the results of staining the remaining extracellular matrix with BCIP after peeling cells off with 0.05% EDTA. Panels C and G show the results of color development in the remaining extracellular matrix by addition of a soluble alkaline phosphatase substrate (PNPP) thereto after peeling cells off with 0.05% EDTA. Panels D and H show the results of color development reaction by addition of PNPP to the cell culture medium (culture supernatant) in the same manner as in conventional methods.

Those sites stained purple are alkaline phosphatase activity sites, i.e., the deposition sites of 4-1 (E and F). From the results shown in E and F in Fig. 2, it is found that 4-1 deposited onto cells and extracellular matrix. On the other hand, Del-1 minor did not deposit either cells or extracellular matrix (A and B).

Likewise, extracellular matrix was stained yellow with the soluble substrate PNPP (G) when 4-1 was used, but extracellular matrix was not stained at all when Del-1 minor was used (C). Further, when PNPP was added to cell culture medium for color development reaction, the culture medium was stained yellow when Del-1 minor was used (D) but no color development was observed when 4-1 was used (H). Therefore, it has been found that 4-1 deposited onto extracellular matrix but Del-1 minor deposited little.

In the present invention, it is possible to measure the alkaline phosphatase activity in extracellular matrix with an absorptionmeter or the like by allowing the substrate of alkaline phosphatase to develop a color using soluble alkaline phosphatase as shown in G in

Fig. 2.

Then, the inventor measured alkaline phosphatase activities in extracellular matrix and cell culture medium on the Del-1 partial fragment (4-1) and the full-length Del-1, and compared them. The results revealed that the Del-1 partial fragment (4-1) has 2.5-fold higher deposition activity onto extracellular matrix than the full-length Del-1.

(3) A truncated *Del-1* gene sequence (XY) as shown in SEQ ID NO: 17 (one of the *Del-1* partial fragments prepared in Example 1) was ligated to alkali phosphatase gene, and the resultant DNA (AP/XY) was introduced into mouse livers. As a control, mouse livers into which alkali phosphatase gene (AP) alone was introduced were prepared. Twenty-four hours after the gene transfer, plasma and hepatic tissues were taken from individual livers, followed by measurement of alkaline phosphatase activities.

In the above-gene transfer, β-galactosidase gene was introduced simultaneously with the above-mentioned AP/XY or AP in order to standardize the efficiency of gene transfer. β-Galactosidase activity was also measured together with alkaline phosphatase activity. The quotient obtained by dividing the measured alkaline phosphatase activity by the value of β-galactosidase activity was taken as the measured value (AP/Lac ratio). Further, AP/Lac ratio in the plasma or hepatic tissue taken from livers of those mice into which the DNA composed of XY and alkaline phosphatase gene ligated (AP/XY) was introduced is shown in graphs, taking the corresponding AP/Lac ratio in control mouse into which alkaline phosphatase gene (AP) alone was introduced as "1". Fig. 3 shows AP/Lac ratios in the plasma taken from individual livers. Fig. 4 shows AP/Lac ratios in the hepatic tissues taken from individual livers.

With respect to AP/Lac ratio in hepatic tissues, hepatic tissues taken from AP/XY-introduced livers showed about 8-fold higher AP/Lac ratio than hepatic tissues taken from AP alone introduced livers (Fig. 4). On the other hand, with respect to AP/Lac ratio in plasma, AP activity was hardly detected in the plasma taken from AP/XY-introduced livers and, thus, the AP/Lac ratio was almost 0.

(4) Three cryosections were prepared from AP/XY-introduced mouse livers prepared in (3) (B, E and F). Similarly, three cryosections were prepared from AP alone introduced mouse livers (A, C and D).

Fig. 5 shows the results of alkaline phosphatase staining (A, B, C and E) and β-galactosidase staining (D and F) on the cryosections of hepatic tissues taken from individual livers. A and B were observed at x40 magnification, and C, D, E and F at x200

magnification. Compared to AP (cryosections A), AP/XY (cryosections B) deposits remarkably. Cryosections C and D and cryosections E and F were serial sections, respectively, and stained with both alkaline phosphatase and β-galactosidase staining. AP (cryosections C and D) is also stained with β-galactosidase staining (cryosections D and F) in the same manner as seen in AP/XY (cryosections E and F). This indicates that there is no difference in gene transfer efficiency.

(5) Subsequently, the full-length Del-1 and the Del-1 partial fragment XY prepared in Example 1 were detected by Western blotting. Specifically, the three genes described below were prepared and introduced into cos7 cells.

(i) a DNA in which the full-length *Del-1* gene sequence as shown in SEQ ID NO: 1 (Del-1 major) and alkaline phosphatase gene are ligated (AP/Del-1)

(ii) a DNA in which the truncated *Del-1* gene sequence as shown in SEQ ID NO: 17 (XY) and alkaline phosphatase gene are ligated (AP/XY)

(iii) as a control, alkaline phosphatase gene alone (AP)-introduced cos7 cells were prepared; and cos7 cells without gene transfer (NC) were also prepared.

Subsequently, the above-described four types of cos7 cells were cultured individually for 72 hours. Then, the culture medium and extracellular matrix (ECM) were collected and subjected to Western blotting. As controls, laminin and albumin were used.

Fig. 6 is photographs showing the results of electrophoresis in the Western blotting. The upper photograph shows electrophoresis using laminin as a control. The lower photograph shows electrophoresis using albumin as a control.

According to Fig. 6, when AP alone introduced cos7 cells were used, the recombinant protein of alkaline phosphatase was not detected in extracellular matrix, as seen in the case of cos7 cells without gene transfer (NC). However, the recombinant protein was detected in the medium. On the other hand, when AP/Del-1 or AP/XY introduced cos7 cells were used, the recombinant protein of alkaline phosphatase was detected highly in extracellular matrix.

[EXAMPLE 3] Recovery of Molecules of Interest

This Example illustrates an example in which alkaline phosphatase is recovered as the expression product from a gene of interest. The recovery of alkaline phosphatase was confirmed by detecting the color development reaction of alkaline phosphatase with its substrate.

Briefly, a DNA in which alkaline phosphatase gene and a truncated *Del-1* gene

sequence (4-1) are ligated was introduced into cos7 cells. As controls, wild-type cos7 cells and alkaline phosphatase gene alone introduced cos7 cells were also prepared.

These cells were cultured for 3 days. Then, the cells were removed with 0.05% EDTA solution, and the extracellular matrix remaining on the bottom of the culture dish was recovered with a scraper. The thus recovered sample was centrifuged and the resultant supernatant was removed to thereby prepare pellet. Subsequently, the same operations as in Example 2 (Fig. 3, B and F) were performed, and BCIP (substrate of alkaline phosphatase) was added to the pellet for color development.

The results are shown in Fig. 7. In Fig. 7, panel (a) shows the results in wild-type cos7 cells; panel (b) shows the results in alkaline phosphatase gene alone introduced cos7 cells; and panel (c) shows the results in the fusion gene (4-1 partial fragment + alkaline phosphatase gene) introduced cos7 cells. As shown previously in Fig. 7, in sample (c) into which a *Del-1* partial fragment (4-1) was introduced, the pellet was stained dark blue purple. This demonstrates that alkaline phosphatase was recovered into insoluble extracellular matrix through the Del-1 partial fragment (4-1). In contrast, color development was hardly observed in control cells, indicating that little alkaline phosphatase was recovered.

INDUSTRIAL APPLICABILITY

By using the Del-1 partial fragment of the present invention, it is possible to allow a molecule of interest to deposit onto extracellular matrix or artificial materials efficiently. The Del-1 partial fragment of the present invention is also useful in recovering or removing a molecule of interest by means of the above-mentioned deposition. According to the present invention, by using the Del-1 partial fragment, it is possible to allow a molecule of interest to deposit onto extracellular matrix to thereby prevent the flow out of the molecule into plasma highly. Thus, a fusion protein having the Del-1 partial fragment of the invention and the molecule of interest may be used as a drug delivery system with less side effect. Further, by regulating deposition activity with the Del-1 partial fragment of the invention, it is possible to highly control the degree of concentration at a specific site or localization of the molecule of interest. Thus, such a fusion protein may be used as an extremely highly functional drug delivery system.